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Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis

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We have generated transgenic mouse lines carrying and expressing wild-type and 3'-modified human tumour necrosis factor (hTNF-α, cachectin) transgenes. We show that correct, endotoxin-responsive and macrophage-specific hTNF gene expression can be established in transgenic mice and we present evidence that the 3'-region of the hTNF gene may be involved in macrophage-specific transcription. Transgenic mice carrying 3'-modified hTNF transgenes shows deregulated patterns of expression and interestingly develop chronic inflammatory polyarthritis. Treatment of these arthritic mice with a monoclonal antibody against human TNF completely prevents development of this disease. Our results indicate a direct involvement of TNF in the pathogenesis of arthritis. Transgenic mice which predictably develop arthritis represent a novel genetic model by which the pathogenesis and treatment of this disease in humans may be further investigated.

Key words: arthritis/disease model/gene expression/ TNF/transgenic mice

Introduction

Cytokines play a central role in the regulation of the immune system and they have been implicated in inflammatory processes as well as in the pathogenesis of many diseases (Balkwill and Burke, 1989; Arai *et al.*, 1990). Tumour necrosis factor, first associated with tumour regression and with cachexia accompanying chronic invasive diseases, is now established as an immune modulator in normal and chronic inflammatory situations (reviewed by Beutler and Cerami, 1989; Old, 1990). TNF is mainly a product of activated macrophages. Other cell types known to synthesize TNF at much lower levels include activated T cells (Steffen *et al.*, 1988; Kinkhabwala *et al.*, 1990), natural killer cells (Peters *et al.*, 1986) and mast cells (Gordon and Galli, 1990). In contrast, almost every cell type is found to express a TNF-specific plasma membrane receptor (Loetscher *et al.*, 1990; Schall *et al.*, 1990). TNF production is under strict regulation which is achieved mainly at the post-transcriptional level. Thus, in resting macrophages, low levels of TNF mRNA can be detected while no protein is synthesized (Beutler *et al.*, 1986). Following activation by lipopolysaccharide (LPS), a major inducer of inflammation, transcription from the TNF gene is augmented 3-fold while steady-state TNF mRNA levels are increased by 50-fold or more (Beutler *et al.*, 1986). In addition, TNF production is further regulated by LPS at the translational level (Han *et al.*, 1990). Deregulated production of TNF in humans is thought to contribute to the development of diseases such as cancer-associated cachexia (Oliff *et al.*, 1987), endotoxic shock (Beutler *et al.*, 1985), graft versus host disease (Piguet *et al.*, 1987), autoimmunity (Held *et al.*, 1990) and rheumatoid arthritis (Saxne *et al.*, 1988; Yocum *et al.*, 1989).

Rheumatoid arthritis (RA) and other inflammatory arthritides are chronic disorders with poorly understood aetiology and pathogenesis (Harris, 1990). Inflammation is localized in the synovial lining, a monolayer of synovial cells that lines diarthroidal joints. In arthritis, the synovial lining becomes markedly thickened due to synovial cell proliferation and infiltration by inflammatory cells. This proliferative mass, the pannus, invades and destroys articular cartilage and bone, leading to irreversible destruction of joint structure and function. The proliferation of the synovial cells and the formation of the characteristic pannus is most probably the key factor leading to the destruction of connective tissue and subchondral bone in arthritis. Several recent studies have implicated cytokines in the pathogenesis of this disease. For example, consistently elevated levels of TNF (Saxne *et al.*, 1988; Di Giovine *et al.*, 1988) as well as interleukin 1 (Nouri *et al.*, 1984) and interleukin 6 (Hirano *et al.*, 1988) are found in the synovial fluid of RA patients. In addition, it has been shown that synovial cells are triggered to proliferate by rTNF and rIL-1 *in vitro* (Butler *et al.*, 1988; Gitter *et al.*, 1989). Moreover, it has been suggested that TNF and interleukin 1 are involved in the process of cartilage destruction and bone resorption since they induce collagenase production by synovial cells (Dayer *et al.*, 1985; Dayer *et al.*, 1986), inhibit proteoglycan synthesis by articular chondrocytes (Saklatvala, 1986; Saklatvala *et al.*, 1985) and stimulate bone resorption *in vitro* (Bertolini *et al.*, 1986; Gowen *et al.*, 1983).

To further our understanding of the mechanisms regulating TNF gene expression and to evaluate the effects of constitutive and abnormal TNF production *in vivo*, we generated several transgenic mouse lines carrying and expressing wild-type and 3'-modified human TNF gene constructs. We show that endotoxin-responsive expression of a hTNF transgene can be established in transgenic mice and that the necessary *cis*-acting DNA information for this is contained within a 3.6 kb DNA fragment. In addition, our results indicate that the 3'-region of the hTNF gene is necessary for macrophage-specific expression. Interestingly, transgenic mice expressing 3'-modified hTNF transgenes develop chronic inflammatory polyarthritis. Furthermore, development of arthritis can be prevented by the *in vivo*

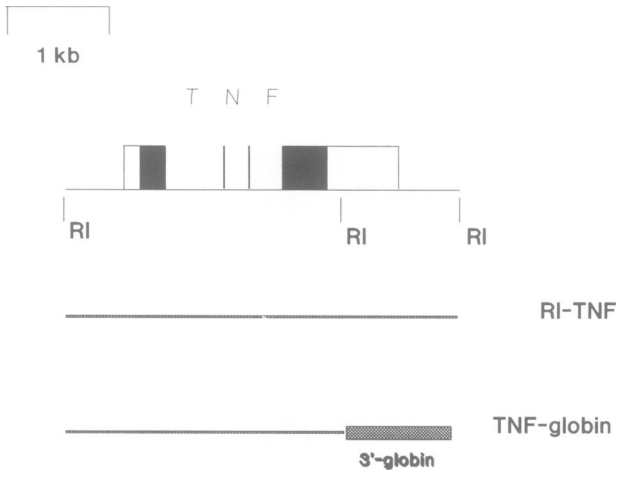

Fig. 1. Structure of the human TNF gene (Nedwin *et al.*, 1985) and the TNF gene constructs which were microinjected into mouse zygotes (see also Materials and methods). Open boxes denote 5′- and 3′-untranslated regions. Solid boxes denote coding regions.

administration of monoclonal antibodies to hTNF. These results provide direct evidence for a role for TNF in the pathogenesis of inflammatory arthritides. Inhibition of this action may prove useful in the design of therapeutic protocols for this group of diseases.

Results

Regulated expression of the human TNF gene in transgenic mice

In order to analyse the expression of the human tumour necrosis factor gene and to understand further its biological function, we developed transgenic mice carrying a 3.6 kb DNA fragment which contains the complete human TNF gene together with 0.6 kb of 5′- and 0.8 kb of 3′-flanking sequences (Figure 1; RI−TNF construct). Microinjection of this fragment into (CBA × C57B1/6) F2 mouse zygotes resulted in three transgenic mouse lines—Tg394, Tg1278 and Tg1281−carrying ~3, 50 and 3 intact copies of the transgene respectively, as assessed by Southern hybridization analysis of DNA from tail biopsies (not shown).

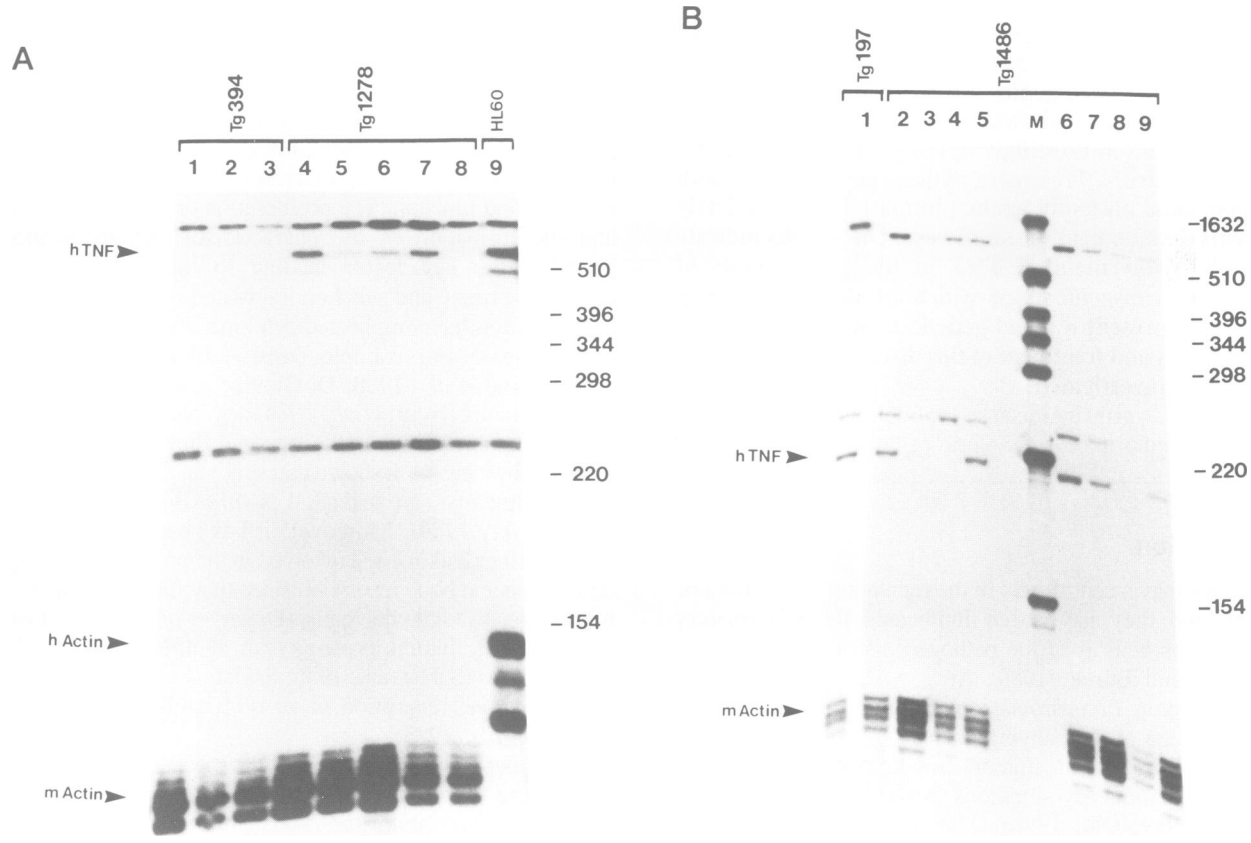

Fig. 2. S1 nuclease protection analysis of total RNA from tissues of transgenic mice carrying RI−TNF and TNF−globin transgenes.
(**A**) Expression analysis in transgenic mice carrying wild-type RI−TNF transgenes. Twenty micrograms of total RNA from spleen, kidney and brain (lanes 1, 2 and 3) of adult Tg394 progeny, and thymus, lung, spleen, kidney and brain (lanes 4−8) of adult Tg1278 progeny was hybridized to a 3′ ^{32}P-end-labelled 0.8 kb *Eco*RI−*Eco*RI probe derived from the 3′-end of the human TNF gene. Correct transcriptional termination of the human TNF gene produces a mRNA that protects 0.6 kb of the probe from S1 nuclease digestion (arrowhead, hTNF). Five micrograms of total RNA from PMA induced human HL-60 cells (lane 9) served as a positive control for human TNF mRNA. (**B**) Expression analysis in transgenic mice carrying 3′-modified (TNF−globin) hTNF transgenes. Twenty micrograms of total RNA from joints (lane 1) of Tg197 progeny at 4 weeks of age, and from joints, gut, liver, brain, kidney, spleen, lung and thymus (lanes 2−9) of founder Tg1486 mouse at 5 weeks of age were hybridized to a 3′^{32}P-end-labelled 0.7 kb *Eco*RI−*Msp*I probe derived from the 3′-end of the human β-globin gene (Kollias *et al.*, 1986). Correct transcriptional termination of the TNF−globin transgene produces a mRNA that protects 212 nt of the probe from S1 digestion (arrowhead, hTNF). A 5′-end labelled β-actin probe was used to control for qualitative and quantitative differences between RNA preparations. Autoradiography was for 65 h at −70°C with an intensifying screen. Sizes were estimated with an end-labelled *Hin*fI digest of pBR322 DNA.

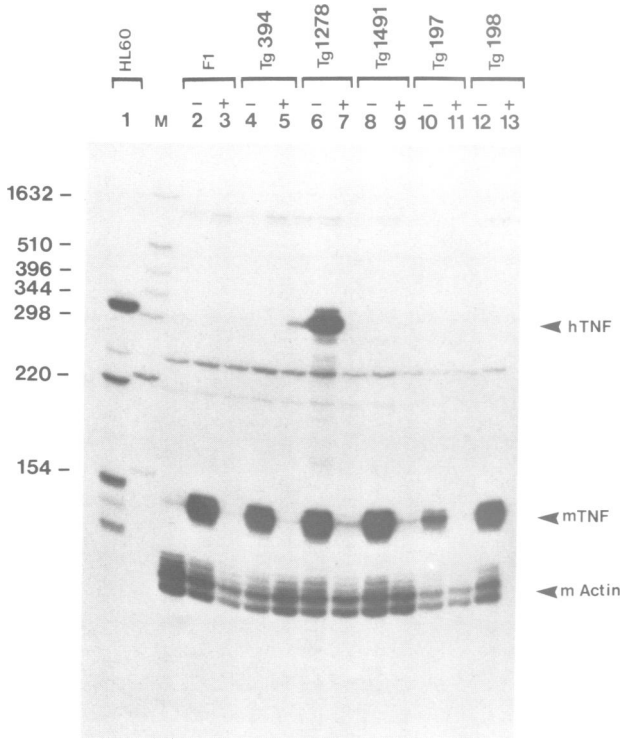

Fig. 3. S1 nuclease protection analysis of total RNA from normal and transgenic, thioglycollate-elicited mouse peritoneal macrophages before (−) and after (+) induction by LPS. Exogenous TNF expression was monitored by a 942 nt 5′-end labelled *Nar*I−*Eco*RI probe derived from the 5′-end of the human TNF gene. Correct initiation of transcription from the human TNF gene produces a mRNA that protects 308 nt of the probe from S1 digestion (arrowhead, hTNF). Endogenous TNF expression was monitored by a 790 nt 5′-end labelled *Nar*I−*Eco*RI probe derived from the 5′-end of the mouse TNF gene (kindly provided by Dr C.V.Jongeneel). Correct initiation of transcription from the mouse TNF gene produces a mRNA that protects 130 nt of the probe from S1 digestion (arrowhead, mTNF). Five micrograms of total RNA from PMA induced human HL-60 cells (lane 1) served as a positive control for human TNF mRNA. A 5′-end labelled β-actin probe was used to control for qualitative and quantitative differences between RNA preparations. Autoradiography was for 65 h at −70°C with an intensifying screen. Sizes were estimated with an end-labelled *Hin*fI digest of pBR322 DNA.

All three founder mice and their progeny developed normally, with the exception of an inherited and not yet characterized skin lesion phenotype observed in line Tg1278. This lesion did not appear to affect normal development. Gross and histological examination of Tg1278 progeny did not reveal further abnormalities. As was expected from the absence of pathological changes, TNF bioactivity was undetectable in the serum of these mice when measured by standard L929 cytotoxicity assays (not shown).

G1 and G2 offspring of the three independent transgenic lines were analysed for exogenous TNF mRNA expression both by 5′- and 3′- specific S1 nuclease protection assays. Low level steady-state mRNA specific for human TNF was detected in the thymus, lung, spleen, kidney, brain (Figure 2A, lanes 4−8), joints and skin (data not shown) of transgenic line Tg1278. The other two transgenic lines showed no detectable levels of human TNF mRNA in the same tissues. To assess whether the 3.6 kb human TNF gene fragment, used for the generation of our transgenic lines, contains enough information for LPS-induced macrophage-specific TNF gene expression, we measured the steady-state TNF mRNA levels before and after induction by LPS in thioglycollate-elicited mouse peritoneal macrophages. Using 5′-end specific probes for both mouse and human TNF mRNA, S1 nuclease protection analysis showed a similar pattern of expression and regulation by LPS between exogenous and endogenous TNF genes in the expressing mouse line Tg1278 (Figure 3, lanes 6 and 7).

The above data show that expression of the wild-type hTNF transgene is correctly regulated in transgenic mice and that sufficient information for this is contained within the 3.6 kb fragment which was injected. This system, therefore, can be used to answer questions on the regulation of TNF gene expression and its biological function.

Transgenic mice carrying a 3′-modified hTNF transgene

It is documented that a major level of control on TNF biosynthesis is exerted at the post-transcriptional level (Beutler *et al.*, 1986). A set of highly conserved UA-rich sequences located at the 3′-untranslated region of mRNAs coding for several inflammatory mediators including TNF (Caput *et al.*, 1986) is thought to be critical in the regulation of both mRNA stability (Shaw and Kamen, 1986) and translational efficiency (Han *et al.*, 1990; Kruys *et al.*, 1989). Based on this notion, we reasoned that transgenic mice carrying and expressing TNF gene constructs with a modified 3′-region would constitute a valid *in vivo* model of deregulated TNF gene expression. Such a 3′-modified TNF gene construct was obtained by exchanging the 3′-region of the human TNF gene, containing its 3′-untranslated and 3′-flanking sequences, with that of the human β-globin gene (Fig. 1; TNF−globin gene construct; see also Materials and methods). This modification provides the necessary polyadenylation signal and does not interfere with correct translation of wild-type human TNF protein.

Microinjection of this construct into mouse zygotes resulted in 12 founder transgenic mice. The copy numbers and the integrity of the transgenes were assessed by Southern hybridization analysis of DNA from tail biopsies (not shown). Two of these founders which carried rearranged transgenes and two which did not transmit the transgene were excluded from this study. Of the remaining eight TNF−globin founders, four (Tg173, Tg198, Tg1491 and Tg1495 carrying ~2−5 copies of the transgene) developed normally. The remaining four, however (Tg197, Tg1480, Tg1486 and Tg1577, carrying ~5, 2, 10 and 50 copies of the transgene respectively), developed visible swellings of the ankle joints of both hind paws leading to progressive impairment in movement (see below).

Deregulated expression of the 3′-modified TNF gene in transgenic mice

In order to examine the expression patterns of the TNF−globin transgene, 3′-specific S1 nuclease protection assays were performed on total RNA from several transgenic tissues. Additional 5′-specific S1 assays confirmed correct initiation of transcription from the hTNF transgene (data not shown). Low level exogenous TNF mRNA could be detected in a wide variety of tissues from transgenic lines Tg197 and Tg1486. Figure 2B shows expression of the TNF−globin transgene in thymus, lung, spleen, kidney, brain, liver, gut and joint tissues of founder transgenic mouse Tg1486 (lanes

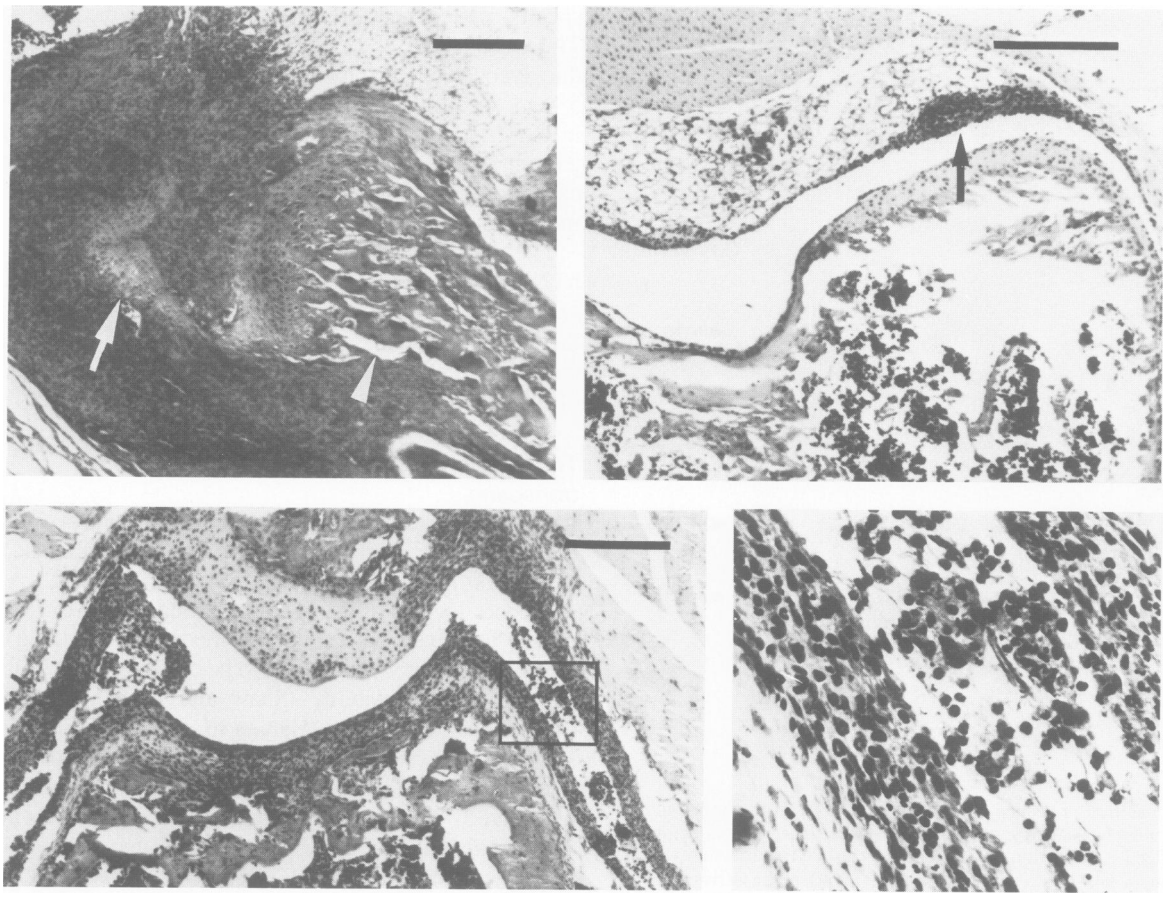

Fig. 4. Histology of peripheral joints showing the progression of arthritis at different developmental stages of Tg197 progeny. (**a**) Synovial hyperplasia and pannus eroding both cartilage (arrow) and bone (arrowhead) in the ankle joint of Tg197-1121 mouse at 4 weeks of age; (**b**) onset of synovial thickening (arrow) in the hip joint of Tg197-1123 mouse at 5 weeks of age; (**c**) hyperproliferation of the synovial layer and massive accumulation of polymorphonuclear cells and lymphocytes in the synovial space of the hip joint of Tg197-710 mouse at 9 weeks of age; (**d**) higher magnification of boxed area in panel (c). Scale bars = 500 μm. Note the differential progression of the disease in distinct joints of transgenic mice. This confirms macroscopic evidence on the progression of arthritis, with obvious swellings consistently developing at 3–4 weeks of age in the ankle joints. Only at later stages does involvement of the other peripheral joints result in the animal's movement being impaired.

2–9) and in joints of Tg197 progeny (lane 1). Expression was also detected at similar levels in brain, thymus, spleen, kidney and lung of the established line Tg197 (data not shown). Transgenic lines Tg173, Tg198, Tg1491 and Tg1495 did not show detectable levels of exogenous TNF mRNA. These results indicate that, as with the wild-type 3.6 kb TNF transgene, low-level constitutive expression can be established in tissues of TNF–globin transgenic mice.

To examine whether the expression of the TNF–globin transgene can be regulated by LPS, peritoneal macrophages from three TNF–globin transgenic lines were assessed for expression of steady-state exogenous TNF mRNA levels before or after induction by LPS (Figure 3, lanes 8–13). In contrast to the results obtained for the Tg1278 transgenic line, no exogenous mRNA signal could be detected in macrophages from the constitutively expressing Tg197 line (Figure 3, lanes 10 and 11). These data suggest that the 3′-region of the human TNF gene, which is deleted in the TNF–globin hybrid gene construct, contains information necessary for macrophage-specific TNF gene expression. Peritoneal macrophages from transgenic lines Tg1491 and Tg198 (Figure 3, lanes 8, 9 and 12, 13) and Tg1495 (not shown) showed no detectable levels of exogenous expression which may be due to integration position effects since no other tissue was found positive for hTNF mRNA.

The 3′-modified TNF transgene induces chronic inflammatory polyarthritis in mice

Breeding of transgenic line Tg197 showed that the macroscopic pathology (swelling of the ankles and impairment in movement) was inherited with a 100% frequency in transgenic progeny. The disease was evident at around 3–4 weeks of age, with swelling of the ankle joints. Impairment in leg movement progressed to complete loss of movement of the hind legs at around 9–10 weeks of age. Moreover, progressive weight loss was a common feature in these mice. Two other affected lines, Tg1480 (which additionally developed wrist swelling) and Tg1486, also maintained subnormal body weights and were sacrificed early for histological and RNA analysis at the age of 3 and 5 weeks, respectively. Weight loss could not be associated with detectable levels of serum TNF in any of these mice, as measured by cytotoxicity assays on mouse L929 cells (not shown). The fourth affected founder, Tg1577, a male with only minor impairment in hind leg movement, failed to breed and was sacrificed at 9 weeks of age for completion of this study.

Histological analyses were performed on affected and unaffected joints from all transgenic lines. Histopathological features of symmetrical polyarthritis were observed only in animals with macroscopic signs of the disease. The develop-

ment of the disease was followed in detail in the progeny of Tg197. Hyperplasia of the synovial membrane (Figure 4B and D) and polymorphonuclear and lymphocytic inflammatory infiltrates of the synovial space (Figure 4C and D) were evident in nearly all joints examined at different developmental stages, starting at 3 weeks of age. In addition, pannus formation, articular cartilage destruction and massive production of fibrous tissue (Figure 4A), all histological characteristics compatible with human rheumatoid arthritis (Trentham, 1982), were observed in the advanced stages of the disease. However, serum levels of rheumatoid factor against total mouse IgG (as determined by direct ELISA, data not shown) were not detectable at any stage of disease.

Histological analysis of several Tg197 mice demonstrated that the onset of arthritis can be placed at around 3–4 weeks of age when synovial thickening is observed. In all cases, swelling of the ankle joints was evident by no more than 5 weeks of age and by that age microscopical findings were showing at least severe hyperplasia and inflammation of the synovium. It is worth noting that histological examination of all other tissues of the TNF–globin transgenic mice did not reveal further abnormalities with the exception of a skin lesion phenotype observed in founder Tg1480 similar to that observed in line Tg1278 (see above).

In vivo administration of monoclonal antibodies against human TNF suppresses the development of arthritis

To obtain direct evidence that the development of arthritis in the Tg197 line occurs as a result of deregulated TNF production, monoclonal antibodies to hTNF were administered intra-peritoneally from birth to ten Tg197 transgenic mice and five non-transgenic littermates. Non-injected transgenic littermates developed macroscopical signs of arthritis by 4–5 weeks of age and showed subnormal body weights, as described above. Antibody-injected animals, however, developed normally and showed no signs of arthritis or weight loss even past 10 weeks of age, when impairment of movement is a common macroscopical feature in these mice (see above). Moreover, transgenic progeny of antibody-treated animals developed arthritis within the usual time course.

Histological analysis of joints was performed in antibody-injected and non-injected transgenic and non-transgenic littermates sacrificed at 5 and 8 weeks of age. As expected, joints from non-injected transgenic mice already showed synovial thickening and inflammatory cell infiltration at 5 weeks of age (Figure 5C). Joints from antibody-treated animals, however, were normal in appearance at both 5 (data not shown) and 8 weeks of age (Figure 5B) and matched those of non-transgenic control animals sacrificed at the same age (Figure 5A).

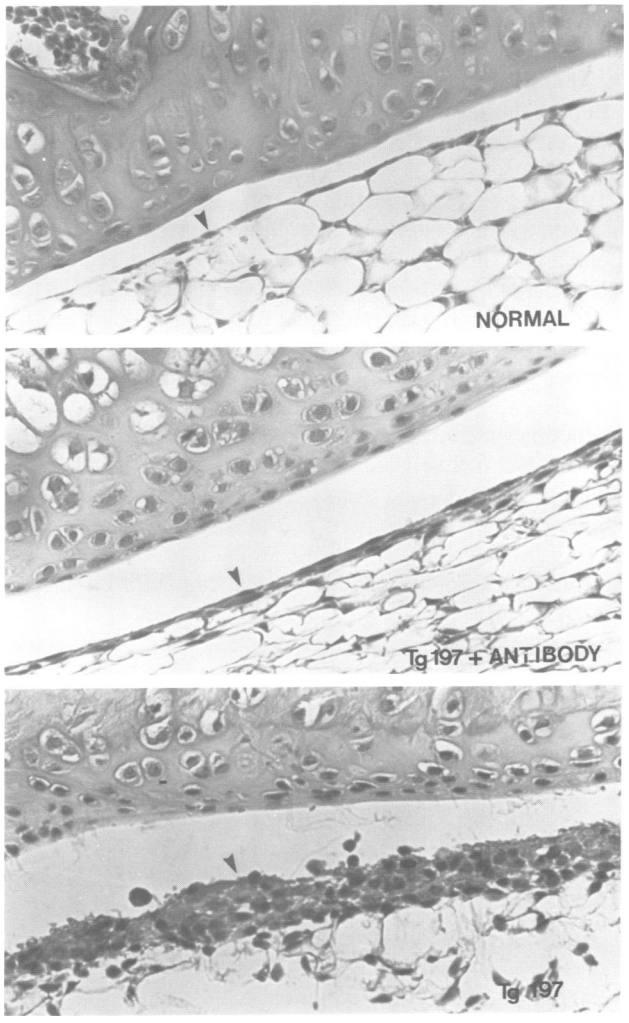

Fig. 5. Suppression of arthritis in monoclonal antibody treated Tg197 mice. Sections through the knee joints of normal (**A**), monoclonal antibody treated Tg197 progeny (**B**) and non-treated Tg197 progeny (**C**). Thickening of the synovial layer (arrowheads) is completely suppressed in the antibody treated transgenic mice.

Discussion

We have studied the expression and biological role of tumour necrosis factor by introducing wild-type and 3′-modified human TNF gene constructs into the germ line of mice. First, we show that low level, endotoxin-responsive expression of a wild-type human TNF gene can be established in transgenic mice and that the necessary *cis*-acting sequences for this are contained within a 3.6 kb DNA fragment. These mice develop normally with undetectable TNF bioactivity in their serum, suggesting that the regulation of expression of the exogenous gene is similar to that of the endogenous gene. Macrophages from transgenic mice carrying a 3′-modified TNF gene construct do not express the transgene and this suggests a direct role for this 3′-region in the macrophage-specific accumulation of TNF mRNA. Whether this is due to interference at the transcriptional level or in mRNA stability remains to be determined.

Transgenic mice carrying this 3′-modified human TNF gene show deregulated patterns of hTNF gene expression and develop chronic inflammatory polyarthritis. Our finding that development of arthritis in these transgenic mice can be completely suppressed by treatment with antibodies against human TNF confirms that the pathology observed is effected by the deregulated production of TNF protein *in vivo*. The mode of TNF deregulation which leads to the development of arthritis in the TNF–globin transgenic mice remains unclear. Although exogenous TNF transcripts are readily detectable in joint tissues from transgenic mice carrying both wild-type and 3′-modified TNF gene constructs, it is possible that in each case different cell types are responsible for the observed expression. Alternatively,

the observed pathology may be due to a quantitative difference in the expression of TNF resulting from the introduced 3'-modification. This interpretation is consistent with data supporting a role for this 3'-region in the translational efficiency of TNF mRNA (Han *et al.*, 1990; Kruys *et al.*, 1989).

Tumour necrosis factor is produced by activated macrophages and is involved in the inflammatory response (Beutler and Cerami, 1989). In fact, it has been documented that a source of TNF production in the inflamed joint is the infiltrating macrophage (Husby *et al.*, 1988; Yocum *et al.*, 1989). The absence of detectable mRNA in peritoneal macrophages from the TNF−globin arthritic mice indicates that this cell type may not be the only source of TNF production in arthritis. Chondrocytes have been shown to share several functional similarities with macrophages (Tiku *et al.*, 1985, 1990) and to be capable of cytokine secretion (Ollivierre *et al.*, 1986). Our preliminary data using *in situ* hybridization analysis of arthritic joints from the Tg197 line indicate that articular chondrocytes can express hTNF mRNA.

The role of cytokines in the pathogenesis of rheumatoid arthritis and other inflammatory arthritides has gained much attention recently (Houssiau and Nagant de Deuxchaisnes, 1990). Several studies have shown that TNF and other monocyte-derived cytokines play an important role in the cascade of events leading to the irreversible destruction of articular cartilage in arthritis (see Introduction). Probably the most striking piece of evidence is that cytokines drive the proliferation of synovial cells *in vitro* (Butler *et al.*, 1988; Gitter *et al.*, 1989). However, the causative factor in arthritis is still unknown. In general, it is believed that either exogenous infectious agents or endogenous factors (i.e. collagen, altered immunoglobulins, histocompatibility epitopes) are initiating the inflammatory, immune and autoimmune processes which lead to the development of arthritis (Harris, 1990). Irrespective of the primary aetiology of arthritis, we provide *in vivo* evidence that deregulation of TNF production *per se* triggers the development of this complex disease. Conceivably, the use of inhibitors of this specific TNF action may serve in the design of protocols aiming at prevention or therapy in this group of diseases.

The genetic mouse model we present in this study complements already existing experimentally induced (Jasin, 1988; Ridge *et al.*, 1988a,b; Taurog *et al.*, 1988; Wooley, 1988) and spontaneous (Hang *et al.*, 1982; Hammer *et al.*, 1990) animal models of human arthritis. Furthermore, it constitutes the first predictive animal model for arthritis, a property expected to facilitate greatly further experimentation on its pathogenesis and treatment.

Materials and methods

Generation of transgenic mice

Original inbred CBA, C57B1/6 and outbred NMRI mice were obtained from IFFA-CREDO and maintained in this institute's facility. CBA × C57B1/6 hybrid mice (F1) were used for matings throughout the study. NMRI vasectomised males were used to produce pseudopregnant F1 females for oviduct transfers. To obtain fertilized F2 zygotes for microinjection, 3-week old F1 females were superovulated as described (Hogan *et al.*, 1986) and mated with F1 males. Microinjection was performed essentially as described elsewhere (Brinster *et al.*, 1985; Kollias *et al.*, 1986). Viable eggs were transferred the same day into oviducts of pseudopregnant females and allowed to develop to term.

To identify transgenic founder mice, DNA was isolated from tail biopsies at 10−15 days of age, digested with *Eco*RI and subjected to Southern blot analysis (Southern, 1975). The integrity of the transgenes was verified by this technique and copy numbers were estimated by comparison of signal intensity to that of the endogenous Thy-1 single copy gene. Transgenic progeny from the founder mice was identified by either Southern or slot blot hybridization analysis.

Preparation of hTNF gene constructs

The human TNF genomic sequences used throughout this study were cloned by Kioussis *et al.* (1987). The 3.6 kb wild-type human TNF gene was subcloned into the *Eco*RI site of the pBluescript vector (Stratagene) and excised for microinjection as a *Bam*HI−*Sal*I fragment. The human TNF/β-globin (TNF−globin) recombinant gene construct was prepared in the pBluescript vector as follows: The 2.8 kb *Eco*RI fragment which contains the entire coding region of the human TNF gene was ligated to the 0.77 kb *Eco*RI−*Sal*I fragment which contains the 3'-untranslated region and polyadenylation site of the human β-globin gene (Kollias *et al.*, 1987) and excised for microinjection as a *Bam*HI−*Sal*I fragment. DNA fragments for microinjection were purified from vector sequences by preparative electrophoresis in low gelling temperature agarose (FMC), followed by passage through an Elutip-D column (Schleicher & Schuell). They were finally dissolved for microinjection in 10 mM Tris−HCl pH 7.6, 0.1 mM EDTA to a final concentration of 2 μg/ml.

RNA analysis

Total RNA was extracted from mouse tissues using the lithium chloride−urea method (Auffray and Rougeon, 1980). After homogenization a 30 s sonication step was included. S1 nuclease protection analysis using human TNF, mouse TNF and human β-actin DNA probes was carried out as described previously (Kollias *et al.*, 1986).

Peritoneal macrophages were harvested by peritoneal lavage from mice injected i.p. 3 days previously with 1 ml of a 4% thioglycollate medium (Difco) and washed once in RPMI plus 10% fetal calf serum. Cells were incubated for 1 h at 37°C, 5% CO_2 for macrophages to adhere to the plastic dish. Non-adhering cells were removed and macrophages were incubated for 1 h in the presence or absence of 1 μg/ml LPS (Sigma).

Histology

Peripheral joints for arthritic and control mice were dissected, immersion-fixed in phosphate-buffered 10% formalin or 4% paraformaldehyde overnight, decalcified in 5% nitric acid for 24−48 h and embedded in Paraplast (BDH). Sections of 5 μm were cut and stained with haematoxylin and eosin according to standard procedures, dehydrated and mounted in DPX (BDH).

TNF monoclonal antibody injections

Litters of progeny from the Tg197 line were divided from birth equally into two groups. The first group received twice weekly i.p. injections of mouse monoclonal antibody to human TNF (provided by Celltech; CB0006) while the second remained untreated. The monoclonal antibody was injected at 8 μg/g of body weight in saline. Both injected and non-injected transgenic littermates and their appropriate non-transgenic controls were sacrificed for histopathological analysis at 5 and 8 weeks of age.

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